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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Daniel E.H. Afar et al.	Examiner:	M. Davis
Serial No.:	09/389,000	Group Art Unit:	1642
Filed:	August 31, 1999	Docket:	G&C 129.27-US-U2
Title:	PHELIX: A TESTIS-SPECIFIC PROTEIN EXPRESSED IN CANCER		

DECLARATION OF RENE S. HUBERT UNDER 37 C.F.R. § 1.132

I, RENE S. HUBERT, declare as follows:

1. I am a named inventor on the patent application identified above, and am authorized by the Assignee to make this declaration.

2. I am a Research Scientist at Agensys, Inc. (formerly known as UroGenesys, Inc.), and hold a B.S. degree in biochemistry from the University of Waterloo, Waterloo, Ontario, and a Ph.D. in molecular biology from the University of Southern California. I served as a post-doctoral research fellow at Cedars-Sinai Medical Center, where I performed genetic research. I have approximately 14 years of experience in scientific research, and have authored at least 15 journal articles in the field of molecular biology.

3. I have reviewed the specification of the above-identified patent application, and find that the specification discloses evidence that the expression of PHELIX polynucleotides is correlated with the expression of PHELIX protein. This evidence can be found in Example 6, at page 36, which demonstrates the expression of a cDNA encoding PHELIX in transfected 293T cells and the detection of PHELIX protein by these transfected cells using antibodies directed against PHELIX protein (Figure 8). In addition, Example 13, at pages 40-41, demonstrates the use of these antibodies to detect PHELIX protein in whole cell lysates and subcellular fractions of PHELIX-expressing cells using Western analysis (Figure 9). This latter Example and the data shown in Figure 9 also confirm the expression of PHELIX in the nuclei as expected for a protein having a nuclear localization signal and a basic helix loop helix structural motif.

4. Further evidence of the exptession of PHELIX protein and the correlation

between the expression of PHELIX polynucleotides and the expression of PHELIX protein is provided in the figure attached herewith. This figure presents immunohistochemical data showing the detection of PHELIX protein in the cytoplasm of 293T cells transfected with an expression vector containing DNA encoding PHELIX, and not in untransfected 293T cells. The data were obtained by testing PHELIX polyclonal antibodies against a cell pellet consisting of 293T cells overexpressing PHELIX from a transfected pcDNA3.1 myc/HIS expression vector (Invitrogen, Carlsbad CA) along with a negative control cell pellet consisting of untransfected 293T cells. The paraffin-embedded cell pellets were sectioned at 4 microns and placed onto positively charged Capillary Gap microscope slides (Ventana Medical Systems, Inc., Tucson, AZ). After dewaxing in xylene, followed by hydration through alcohol series, tissue sections were pretreated in a steamer for 20 minutes in the presence of sodium citrate (10 mM, pH 6.0) in order to optimize antibody reactivity. After cooling for 5 minutes, the slides were immunostained using an ABC-peroxidase technique. Briefly, slides were incubated in blocking serum (5% normal goat serum, 1% BSA for 5 minutes), followed by incubation in 1.25 μg/ml anti-PHELIX polyclonal primary antibody (25 minutes), biotinylated secondary antibody goat-anti-rabbit IgG (25 min) and avidin-biotin complex (ABC) conjugated to peroxidase enzyme (Vector Labs, Burlingame, CA; 25 minutes). Between each incubation, sections were rinsed in PBS. DAB - Diaminobenzidine chromogen (QualTek Molecular Labs) was used to develop the reaction – yielding a brown precipitate. Slides were subsequently counterstained with hematoxylin and then coverslipped. Staining was performed on a TechMate 1000 automated staining instrument (Ventana Medical Systems, Inc., Tucson, AZ) at room temperature. The attached figure shows the detection of PHELIX protein in the 293T cells expressing PHELIX, but not in the untransfected 293T cells. This specific brown staining shows that PHELIX protein is located within the cytoplasm. These experiments confirm the existence of PHELIX protein, as antibodies produced by immunizing rabbits with a PHELIX peptide specifically stain 293T cells producing the whole PHELIX protein.

5. The evidence referred to in paragraphs 3 and 4 above is consistent with the general knowledge in the art of molecular biology that, with rare exceptions, expression of a polynucleotide, particularly mRNA with an open reading frame and a Kozak consensus sequence for translation initiation, is predictive of expression of the corresponding protein.

6. Based on the above-identified application's disclosure of the highly restricted pattern of mRNA expression, wherein PHELIX is not expressed in normal tissues, except for testis, and is highly expressed in certain cancers, including prostate cancer, PHELIX protein is expected to be useful as a diagnostic and therapeutic tool for the detection and treatment of cancers expressing PHELIX.

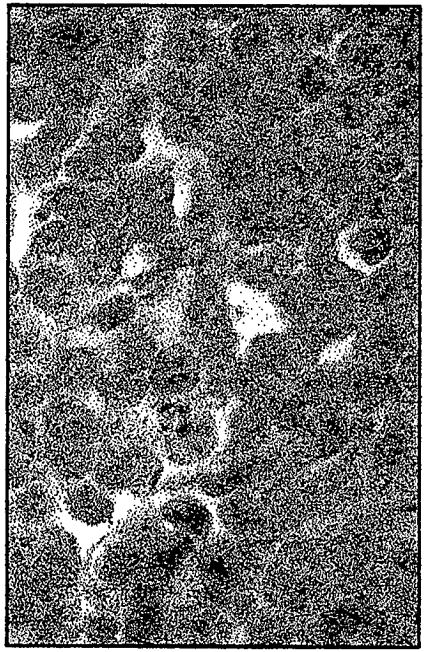
7. Given the disclosure in the specification and the knowledge in the art, one can readily identify, prepare and use PHELIX proteins, PHELIX fragments and other PHELIX polypeptides using conventional techniques. In particular, useful PHELIX polypeptides and fragments can be identified as having the ability to elicit antibody production or to bind MHC class I or II molecules and be recognized by a T cell that specifically recognizes PHELIX protein through the use of standard laboratory protocols. Protocols for producing and identifying useful PHELIX fragments and polypeptides can be modeled after those presented in Examples 5 and 6 of the specification, at pages 35-36, which demonstrate the production of a fragment of PHELIX protein and its ability to elicit the production of antibodies in rabbits.

8. I further declare that all statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 7-16-01

Rene S. Hubert, Ph.D.

PHELIX Expression in Transfected 293T Cells



293T

293T-PHELIX